an·ky·losed (ang'ki-lōsd). Stiffened; bound by adhesions; denoting a joint in a state of ankylosis.

an·ky·lo·sis (ang'ki-lō'sis). Stiffening or fixation of a joint as the result of a disease process, with fibrous or bony union across the joint. [G. *ankylōsis,* stiffening of a joint]

artificial a., SYN arthrodesis.

bony a., SYN synostosis.

dental a., bony union of the radicular surface of a tooth to the surrounding alveolar bone in an area of previous partial root resorption.

extracapsular a., stiffness of a joint due to induration or heterotopic ossification of the surrounding tissues. SYN spurious a.

false a., SYN fibrous a.

fibrous a., stiffening of a joint due to the presence of fibrous bands between and about the bones forming the joint. SYN false a., pseudankylosis.

intracapsular a., stiffness of a joint due to the presence of bony or fibrous adhesions between the articular surfaces of the joint.

spurious a., SYN extracapsular a.

true a., SYN synostosis.

An·ky·los·to·ma (ang-ki-los'tō-mă). **1.** SYN *Ancylostoma.* **2.** SYN trismus. [ankylo- + G. *stoma,* mouth]

an·ky·lo·sto·mi·a·sis (ang'ki-lō-stō-mī'ă-sis). SYN ancylostomiasis.

an·ky·lot·ic (ang-ki-lot'ik). Characterized by or pertaining to ankylosis.

an·ky·rin (ang'ki-rin). An erythrocyte membranal protein that binds spectrin. A deficiency in ankyrin may lead to a type of hereditary spherocytosis. SYN anchorin, syndein. [G. *ankyra,* anchor, + -in]

an·ky·roid (an'ki-royd). SYN ancyroid.

an·la·ge, pl. **an·la·gen** (ahn'lah-ge, -gen). **1.** SYN primordium. **2.** In psychoanalysis, genetic predisposition to a given trait or personality characteristic. [Ger. plan, outline]

an·neal (an-nēl'). **1.** To soften or temper a metal by controlled heating and cooling; the process makes a metal more easily adapted, bent, or swaged, and less brittle. **2.** In dentistry, to heat gold leaf preparatory to its insertion into a cavity, in order to remove adsorbed gases and other contaminants. **3.** The pairing of complementary single strands of DNA; or of DNA-RNA. **4.** The attachment of the ends of two macromolecules; *e.g.,* two microtubules annealing to form one longer microtubule. **5.** In molecular biology, annealing is a process in which short sections of single-stranded DNA from one source are bound to a filter and incubated with single-stranded, radioactively conjugated DNA from a second source. Where the two sets of DNA possess complementary sequences of nucleotides, bonding occurs. The degree of relatedness (homology) of the two sets of DNA is then estimated according to the radioactivity level of the filter. This technique plays a central role in the classification of bacteria and viruses. SYN nucleic acid hybridization. [A.S. *anaelan,* to burn]

an·nec·tent (a-nek'tent). Connected with; joined. [L. *an-necto,* pres. p. *-nectnes,* pp. *-nexus,* to join to]

An·nel·i·da (an'nĕ-lī'dă). A phylum that includes the segmented or true worms, such as the earthworm.

an·ne·lids (an'nĕ-lids). Common name for members of the phylum Annelida.

an·nel·lide (an'ĕ-līd). A conidiogenous cell that produces conidia in succession, each leaving a ringlike collar on the cell wall when released. [Fr. *annelide,* fr. L. *anellus,* a ring]

an·nel·lo·co·nid·i·um (an'ĕ-lō-kŏ-nid'ē-um). A conidium produced by an annellide.

an·nexa (a-nek'să). SYN adnexa.

an·nex·al (a-neks-ăl). SYN adnexal.

an·nex·ec·to·my (an-eks-ek'tō-mē). SYN adnexectomy.

an·nex·ins (a-nek'sinz). A family of Ca^{2+}dependent phospholipid-binding proteins which may act as mediators of intracellular calcium signals.

an·nex·i·tis (an-eks-ī'tis). SYN adnexitis.

an·nex·o·pexy (an-eks'ō-pek-sē). SYN adnexopexy.

an·not·to (ă-not'ō). Coloring matter extracted from the seeds of *Bixa orellana;* contains bixin and several other yellow to orange-red pigments; used for coloring butter, margarine, cheese, and oils.

an·nu·lar (an'yū-lăr). Ring-shaped. SYN anular. [L. *anulus,* ring]

an·nu·lo·plasty (an'yū-lō-plas-tē). Reconstruction of the ring (or annulus) of an incompetent cardiac valve. [L. *anulus,* ring, + G. *plastos,* formed]

an·nu·lor·rha·phy (an-yū-lōr'ă-fē). Closure of a hernial ring by suture. [L. *anulus,* ring, + G. *rhaphē,* seam]

an·nu·lus (an'yū-lŭs) [NA]. SYN ring. SEE ALSO ring.

a. abdomina'lis, SYN deep inguinal *ring.*

a. cilia'ris, SYN ciliary *body.*

a. conjuncti'vae [NA], SYN conjunctival *ring.*

a. femora'lis [NA], SYN femoral *ring.*

a. fibrocartilagin'eus membranae tympani [NA], SYN fibrocartilaginous *ring* of tympanic membrane.

a. fibro'sus [NA], **(1)** SYN fibrous *ring* of heart. **(2)** SYN a. fibrosus of intervertebral disc.

a. fibrosus cordis [NA], SYN fibrous *ring* of heart.

a. fibrosus disci intervertebralis [NA], SYN a. fibrosus of intervertebral disc.

a. fibrosus of intervertebral disc, the ring of fibrocartilage and fibrous tissue forming the circumference of the intervertebral disc; surrounds the nucleus pulposus, which is prone to herniation when the a. fibrosus is compromised. SYN a. fibrosus disci intervertebralis [NA], a. fibrosus (2) [NA], fibrous ring of intervertebral disc, fibrous ring (2).

a. of fibrous sheath, SYN annular *part* of fibrous digital sheath.

Haller's a., SYN Haller's *insula.*

a. hemorrhoida'lis, SYN hemorrhoidal *zone.*

a. inguina'lis profun'dus [NA], SYN deep inguinal *ring.*

a. inguina'lis superficia'lis [NA], SYN superficial inguinal *ring.*

a. ir'idis, SYN *ring* of iris.

a. iridis major, SYN greater *ring* of iris.

a. iridis minor, SYN lesser *ring* of iris.

a. lymphat'icus car'diae [NA], SYN lymphatic *ring* of cardiac part of stomach.

a. ova'lis, SYN *limbus* fossae ovalis.

a. tendin'eus commu'nis [NA], SYN common tendinous *ring.*

a. tympan'icus [NA], SYN tympanic *ring.*

a. umbilica'lis [NA], SYN umbilical *ring.*

a. urethra'lis, SYN *sphincter* vesicae.

Vieussens' a., SYN *limbus* fossae ovalis.

AnOC Abbreviation for anodal opening *contraction.*

an·o·chle·sia (an'ō-klē'zē-ă). **1.** SYN catalepsy. **2.** Quietude. [G. *an-* priv. + *ochlēsis,* disturbance]

an·o·chro·ma·sia (an'ō-krō-mā'zē-ă). **1.** Failure of cells or other elements of tissue to be colored in the usual manner when treated with a stain (or stains). **2.** Accumulation of hemoglobin in the peripheral zone of erythrocytes, thereby resulting in a pale, virtually colorless central portion. [G. *anō,* upward, + *chrōma,* color]

ano·ci·as·so·ci·a·tion (ă-nō'sē-ă-sō-sē-ā'shŭn). Theory that afferent stimuli, especially pain, contribute to the development of surgical shock, and, as a corollary, that conduction anesthesia at the surgical field and pre-surgical sedation protect against shock. [G. *a-* priv. + L. *noceo,* to injure, + association]

ano·coc·cyg·e·al (a-nō-kok-sij'ē-ăl). Relating to both anus and coccyx.

anod·al (an-ōd'ăl). Of, pertaining to, or emanating from an anode. SYN anodic.

an·ode (an'ōd). **1.** The positive pole of a galvanic battery or the electrode connected with it; an electrode toward which negatively charged ions (anions) migrate; a positively charged electrode. Cf. cathode. **2.** The portion, usually made of tungsten, of an x-ray tube from which x-rays are re leased by bombardment by cathode rays (electrons). SYN anelectrode, positive electrode. [G. *anodos,* a way up, fr. *ana,* up, + *hodos,* a way]

rotating a., in diagnostic radiography, modern x-ray tubes that

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of Law will be entered on the same date herewith.

ORDER AND JUDGMENT

In accordance with the Findings of Fact and Conclusions of Law entered on the same date herewith,

IT IS HEREBY ORDERED AND ADJUDGED, as follows:

1. The Nolan patent (No. 4,506,189), issued on March 19, 1985, is a valid patent.

2. By the manufacture, production, sale and distribution of its SAF-T-COTE fluorescent lamp, Trojan has infringed the Nolan patent.

3. By virtue of this infringement, Shat-R-Shield is entitled to injunctive relief. Trojan shall immediately cease and desist from the manufacture, production, sale and distribution of the SAF-T-COTE fluorescent lamp.

4. Trojan shall recall all the SAF-T-COTE fluorescent lamps sold to and still in the possession of its customers.

5. The Court having determined that Trojan's infringement was not willful and wanton, Shat-R-Shield is not entitled to treble damages.

6. Shat-R-Shield shall have no accounting for monetary damages.

7. The Court having found that this is not an exceptional case, Shat-R-Shield is not entitled to its attorney's fees.

8. All claims having been resolved as to all parties herein, this action is now DISMISSED and STRICKEN from the docket.

9. There being no just reason for delay, this is a FINAL and APPEALABLE Order and Judgment.

Court of Appeals, Federal Circuit

In re Wands

No. 87-1454

Decided September 30, 1988

PATENTS

1. Patentability/Validity — Adequacy of disclosure (§115.12)

Data disclosed in application for immunoassay method patent, which shows that applicants screened nine of 143 cell lines developed for production of antibody necessary to practice invention, stored remainder of said cell lines, and found that four out of nine cell lines screened produced antibody falling within limitation of claims, were erroneously interpreted by Board of Patent Appeals and Interferences as failing to meet disclosure requirements of 35 USC 112, since board's characterization of stored cell lines as "failures" demonstrating unreliability of applicants' methods was improper in view of fact that such unscreened cell lines prove nothing concerning probability of success of person skilled in art attempting to obtain requisite antibodies using applicants' methods.

2. Patentability/Validity — Adequacy of disclosure (§115.12)

Disclosure in application for immunoassay method patent does not fail to meet enablement requirement of 35 USC 112 by requiring "undue experimentation," even though production of monoclonal antibodies necessary to practice invention first requires production and screening of numerous antibody producing cells or "hybridomas," since practitioners of art are prepared to screen negative hybridomas in order to find those that produce desired antibodies, since in monoclonal antibody art one "experiment" is not simply screening of one hybridoma but rather is entire attempt to make desired antibody, and since record indicates that amount of effort needed to obtain desired antibodies is not excessive, in view of applicants' success in each attempt to produce antibody that satisfied all claim limitations.

Appeal from decision of Patent and Trademark Office, Board of Patent Appeals and Interferences.

Application for patent of Jack R. Wands, Vincent R. Zurawski, Jr., and Hubert J. P. Schoemaker, serial number 188,735. From decision of Board of Patent Appeals and Interferences affirming rejection of application, applicants appeal. Reversed; Newman, J., concurring in part and dissenting in part in separate opinion.

Jorge A. Goldstein, of Saidman, Sterne, Kessler & Goldstein (Henry N. Wixon, with them on brief), Washington, D.C., for appellant.

John H. Raubitschek, associate solicitor (Joseph F. Nakamura and Fred E. McKelvey, with him on brief), PTO, for appellee.

Before Smith, Newman, and Bissell, circuit judges.

Smith, J.

This appeal is from the decision of the Patent and Trademark Office (PTO) Board of Patent Appeals and Interferences (board) affirming the rejection of all remaining claims in appellant's application for a patent, serial No. 188,735, entitled "Immunoassay Utilizing Monoclonal High Affinity IgM

Antibodies," which was filed September 19, 1980.[1] The rejection under 35 U.S.C. §112, first paragraph, is based on the grounds that appellant's written specification would not enable a person skilled in the art to make the monoclonal antibodies that are needed to practice the claimed invention without undue experimentation. We reverse.

I. *Issue*

The only issue on appeal is whether the board erred, as a matter of law, by sustaining the examiner's rejection for lack of enablement under 35 U.S.C. §112, first paragraph, of all remaining claims in appellants' patent application, serial No. 188,735.

II. *Background*

A. *The Art.*

The claimed invention involves immunoassay methods for the detection of hepatitis B surface antigen by using high-affinity monoclonal antibodies of the IgM isotype. *Antibodies* are a class of proteins (immunoglobulins) that help defend the body against invaders such as viruses and bacteria. An antibody has the potential to bind tightly to another molecule, which molecule is called an antigen. The body has the ability to make millions of different antibodies that bind to different antigens. However, it is only after exposure of an antigen that a complicated *immune response* leads to the production of antibodies against that antigen. For example, on the surface of hepatitis B virus particles there is a large protein called *hepatitis B surface antigen* (HBsAg). As its name implies, it is capable of serving as an antigen. During a hepatitis B infection (or when purified HBsAg is injected experimentally), the body begins to make antibodies that bind tightly and specifically to HBsAg. Such antibodies can be used as reagents for sensitive diagnostic tests (*e.g.*, to detect hepatitis B virus in blood and other tissues, a purpose of the claimed invention). A method for detecting or measuring antigens by using antibodies as reagents is called an *immunoassay*.

Normally, many different antibodies are produced against each antigen. One reason for this diversity is that different antibodies are produced that bind to different regions (determinants) of a large antigen molecule such as HBsAg. In addition, different antibodies may be produced that bind to the same determinant. These usually differ in the tightness with which they bind to the determinant. *Affinity* is a quantitative measure of the strength of antibody-antigen binding. Usually an antibody with a higher affinity for an antigen will be more useful for immunological diagnostic tests than one with a lower affinity. Another source of heterogeneity is that there are several immunoglobulin classes or *isotypes*. Immunoglobulin G (IgG) is the most common isotype in serum. Another isotype, immunoglobulin M (IgM), is prominent early in the immune response. IgM molecules are larger than IgG molecules, and have 10 antigen-binding sites instead of the 2 that are present in IgG. Most immunoassay methods use IgG, but the claimed invention uses only IgM antibodies.

For commercial applications there are many disadvantages to using antibodies from serum. Serum contains a complex mixture of antibodies against the antigen of interest within a much larger pool of antibodies directed at other antigens. There are available only in a limited supply that ends when the donor dies. The goal of monoclonal antibody technology is to produce an unlimited supply of a single purified antibody.

The blood cells that make antibodies are *lymphocytes*. Each lymphocyte makes only one kind of antibody. During an immune response, lymphocytes exposed to their particular antigen divide and mature. Each produces a *clone* of identical daughter cells, all of which secrete the same antibody. Clones of lymphocytes, all derived from a single lymphocyte, could provide a source of a single homogeneous antibody. However, lymphocytes do not survive for long outside of the body in cell culture.

Hybridoma technology provides a way to obtain large numbers of cells that all produce the same antibody. This method takes advantage of the properties of *myeloma* cells derived from a tumor of the immune system. The cancerous myeloma cells can divide indefinitely in vitro. They also have the potential ability to secrete antibodies. By appropriate experimental manipulations, a myeloma cell can be made to fuse with a lymphocyte to produce a single hybrid cell (hence, a hybridoma) that contains the genetic material of both cells. The hybridoma secretes the same antibody that was made by its parent lymphocyte, but acquires the capability of the myeloma cell to divide and grow indefinitely in cell culture. Antibodies produced by a clone of hybridoma cells (i.e., by hybridoma

[1] *In re Wands*, Appeal No. 673-76 (Bd. Pat. App. & Int. Dec. 30, 1986).

cells that are all progeny of a single cell) are called monoclonal antibodies.[2]

B. *The Claimed Invention.*

The claimed invention involves methods for the immunoassay of HBsAg by using high-affinity monoclonal IgM antibodies. Jack R. Wands and Vincent R. Zurawski, Jr., two of the three coinventors of the present application, disclosed methods for producing monoclonal antibodies against HBsAg in United States patent No. 4,271,145 (the '145 patent), entitled "Process for Producing Antibodies to Hepatitis Virus and Cell Lines Therefor," which patent issued on June 2, 1981. The '145 patent is incorporated by reference into the application on appeal. The specification of the '145 patent teaches a procedure for immunizing mice against HBsAg, and the use of lymphocytes from these mice to produce hybridomas that secrete monoclonal antibodies specific for HBsAg. The '145 patent discloses that this procedure yields both IgG and IgM antibodies with high-affinity binding to HBsAg. For the stated purpose of complying with the best mode requirement of 35 U.S.C. §112, first paragraph, a hybridoma cell line that secretes IgM antibodies against HBsAg (the 1F8 cell line) was deposited at the American Type Culture Collection, a recognized cell depository, and became available to the public when the '145 patent issued.

The application on appeal claims methods for immunoassay of HBsAg using monoclonal antibodies such as those described in the '145 patent. Most immunoassay methods have used monoclonal antibodies of the IgG isotype. IgM antibodies were disfavored in the prior art because of their sensitivity to reducing agents and their tendency to self-aggregate and precipitate. Appellants found that their monoclonal IgM antibodies could be used for immunoassay of HbsAg with unexpectedly high sensitivity and specificity. Claims 1, 3, 7, 8, 14, and 15 are drawn to methods for the immunoassay of HBsAg using high-affinity IgM monoclonal antibodies. Claims 19 and 25–27 are for chemically modified (*e.g.*, radioactively labeled) monoclonal IgM antibodies used in the assays. The broadest method claim reads:

> 1. An immunoassay method utilizing an antibody to assay for a substance comprising hepatitis B-surface antigen (HBsAg) determinants which comprises the steps of:
>
> contacting a test sample containing said substance comprising HBsAg determinants with said antibody; and
>
> determining the presence of said substance in said sample;
>
> wherein said antibody is a monoclonal high affinity IgM antibody having a binding affinity constant for said HBsAg determinants of at least 10^9 M^{-1}.

Certain claims were rejected under 35 U.S.C. §103; these rejections have not been appealed. Remaining claims 1, 3, 7, 8, 14, 15, 19, and 25–27 were rejected under 35 U.S.C. §112, first paragraph, on the grounds that the disclosure would not enable a person skilled in the art to make and use the invention without undue experimentation. The rejection is directed solely to whether the specification enables one skilled in the art to make the monoclonal antibodies that are needed to practice the invention. The position of the PTO is that data presented by Wands show that the production of high-affinity IgM anti-HBsAg antibodies is unpredictable and unreliable, so that it would require undue experimentation for one skilled in the art to make the antibodies.

III. *Analysis*

A. *Enablement by Deposit of Micro-organisms and Cell Lines.*

The first paragraph of 35 U.S.C. §112 requires that the specification of a patent must enable a person skilled in the art to make and use the claimed invention. "Patents * * * are written to enable those skilled in the art to practice the invention."[3] A patent need not disclose what is well known in the art.[4] Although we review underlying facts found by the board under a "clearly erroneous" standard,[5] we review enablement as a question of law.[6]

Where an invention depends on the use of living materials such as microorganisms or

[2] For a concise description of monoclonal antibodies and their use in immunoassay see *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1368–71, 231 USPQ 81, 82–83 (Fed. Cir. 1986), *cert. denied*, 107 S.Ct. 1606 (1987).

[3] *W.L. Gore & Assocs., Inc. v. Garlock, Inc.*, 721 F.2d 1540, 1556, 220 USPQ 303, 315 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 851 (1984).

[4] *Lindemann Maschinenfabrik GMBH v. American Hoist & Derrick Co.*, 730 F.2d 1452, 1463, 221 USPQ 481, 489 (Fed. Cir. 1984).

[5] *Coleman v. Dines*, 754 F.2d 353, 356, 224 USPQ 857, 859 (Fed. Cir. 1985).

[6] *Moleculon Research Corp. v. CBS, Inc.*, 793 F.2d 1261, 1268, 229 USPQ 805, 810 (Fed. Cir. 1986), *cert. denied*, 107 S.Ct. 875 (1987); *Raytheon Co. v. Roper Corp.*, 724 F.2d 951, 960 n.6, 220 USPQ 592, 599 n.6 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 835 [225 USPQ 232] (1984).

cultured cells, it may be impossible to enable the public to make the invention (i.e., to obtain these living materials) solely by means of a written disclosure. One means that has been developed for complying with the enablement requirement is to deposit the living materials in cell depositories which will distribute samples to the public who wish to practice the invention after the patent issues.[7] Administrative guidelines and judicial decisions have clarified the conditions under which a deposit of organisms can satisfy the requirements of section 112.[8] A deposit has been held necessary for enablement where the starting materials (i.e., the living cells used to practice the invention, or cells from which the required cells can be produced) are not readily available to the public.[9] Even when starting materials are available, a deposit has been necessary where it would require undue experimentation to make the cells of the invention from the starting materials.[10]

In addition to satisfying the enablement requirement, deposit of organisms also can be used to establish the filing date of the application as the prima facie date of invention,[11] and to satisfy the requirement under 35 U.S.C. §114 that the PTO be guaranteed access to the invention during pendency of the application.[12] Although a deposit may serve these purposes, we recognized, in *In re Lundak*,[13] that these purposes, nevertheless, may be met in ways other than by making a deposit.

A deposit also may satisfy the best mode requirement of section 112, first paragraph, and it is for this reason that the 1F8 hybridoma was deposited in connection with the '145 patent and the current application. Wands does not challenge the statements by the examiner to the effect that, although the deposited 1F8 line enables the public to perform immunoassays with antibodies produced by that single hybridoma, the deposit does not enable the generic claims that are on appeal. The examiner rejected the claims on the grounds that the written disclosure was not enabling and that the deposit was inadequate. Since we hold that the written disclosure fully enables the claimed invention, we need not reach the question of the adequacy of deposits.

B. *Undue Experimentation.*

Although inventions involving microorganisms or other living cells often can be enabled by a deposit,[14] a deposit is not always necessary to satisfy the enablement requirement.[15] No deposit is necessary if the biological organisms can be obtained from readily available sources or derived from readily available starting materials through routine screening that does not require undue experimentation.[16] Whether the specification in an application involving living cells (here, hybridomas) is enabled without a deposit must be decided on the facts of the particular case.[17]

Appellants contend that their written specification fully enables the practice of

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[7] *In re Argoudelis*, 434 F.2d 1390, 1392-93, 168 USPQ 99, 101-02 (CCPA 1970).

[8] *In re Lundak*, 773 F.2d 1216, 227 USPQ 90 (Fed. Cir. 1985); *Feldman v. Aunstrup*, 517 F.2d 1351, 186 USPQ 108 (CCPA 1975), *cert. denied*, 424 U.S. 912 [188 USPQ 720] (1976); Manual of Patent Examining Procedure (MPEP) 608.01 (p)(C) (5th ed. 1983, rev. 1987). *See generally* Hampar, *Patenting of Recombinant DNA Technology: The Deposit Requirement*, 67 J. Pat. Trademark Off. Soc'y 569 (1985).

[9] *In re Jackson*, 217 USPQ 804, 807-08 (Bd. App. 1982) (strains of a newly discovered species of bacteria isolated from nature); *Feldman*, 517 F.2d 1351, 186 USPQ 108 (uncommon fungus isolated from nature); *In re Argoudelis*, 434 F.2d at 1392, 168 USPQ at 102 (novel strain of antibiotic-producing microorganism isolated from nature); *In re Kropp*, 143 USPQ 148, 152 (Bd. App. 1959) (newly discovered microorganism isolated from soil).

[10] *Ex parte Forman*, 230 USPQ 546, 547 (Bd. Pat. App. & Int. 1986) (genetically engineered bacteria where the specification provided insufficient information about the amount of time and effort required); *In re Lundak*, 773 F.2d 1216, 227 USPQ 90 (unique cell line produced from another cell line by mutagenesis).

[11] *In re Lundak*, 773 F.2d at 1222, 227 USPQ at 95-96; *In re Feldman*, 517 F.2d at 1355, 186 USPQ at 113; *In re Argoudelis*, 434 F.2d at 1394-96, 168 USPQ at 103-04 (Baldwin, J. concurring).

[12] *In re Lundak*, 773 F.2d at 1222, 227 USPQ at 95-96; *In re Feldman*, 517 F.2d at 1354, 186 USPQ at 112.

[13] *In re Lundak*, 773 F.2d at 1222, 227 USPQ at 95-96.

[14] *In re Argoudelis*, 434 F.2d at 1393, 168 USPQ at 102.

[15] *Tabuchi v. Nubel*, 559 F.2d 1183, 194 USPQ 521 (CCPA 1977).

[16] *Id.* at 1186-87, 194 USPQ at 525; *Merck & Co. v. Chase Chem. Co.*, 273 F.Supp. 68, 77, 155 USPQ 139, 146 (D.N.J. 1967); *Guaranty Trust Co. v. Union Solvents Corp.*, 54 F.2d 400, 403-06, 12 USPQ 47, 50-53 (D. Del. 1931), *aff'd*, 61 F.2d 1041, 15 USPQ 237 (3d Cir. 1932), *cert. denied*, 288 U.S. 614 (1933); MPEP 608.01(p)(C) ("No problem exists when the microorganisms used are known and readily available to the public.").

[17] *In re Jackson*, 217 USPQ at 807; *see In re Metcalfe*, 410 F.2d 1378, 1382, 161 USPQ 789, 792 (CCPA 1969).

their claimed invention because the monoclonal antibodies needed to perform the immunoassays can be made from readily available starting materials using methods that are well known in the monoclonal antibody art. Wands states that application of these methods to make high-affinity IgM anti-HBsAg antibodies requires only routine screening, and that does not amount to undue experimentation. There is no challenge to their contention that the starting materials (i.e., mice, HBsAg antigen, and myeloma cells) are available to the public. The PTO concedes that the methods used to prepare hybridomas and to screen them for high-affinity IgM antibodies against HBsAg were either well known in the monoclonal antibody art or adequately disclosed in the '145 patent and in the current application. This is consistent with this court's recognition with respect to another patent application that methods for obtaining and screening monoclonal antibodies were well known in 1980.[18] The sole issue is whether, in this particular case, it would require undue experimentation to produce high-affinity IgM monoclonal antibodies.

Enablement is not precluded by the necessity for some experimentation such as routine screening.[19] However, experimentation needed to practice the invention must not be undue experimentation.[20] "the key word is 'undue,' not 'experimentation.' "[21]

> The determination of what constitutes undue experimentation in a given case requires the application of a standard of reasonableness, having due regard for the nature of the invention and the state of the art. Ansul Co. v. Uniroyal, Inc. [448 F.2d 872, 878–79; 169 USPQ 759, 762–63 (2d Cir. 1971), *cert. denied*, 404 U.S. 1018 [172 USPQ 257] (1972)]. The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed * * * .[22]

The term "undue experimentation" does not appear in the statute, but it is well established that enablement requires that the specification teach those in the art to make and use the invention without undue experimentation.[23] Whether undue experimentation is needed is not a single, simple factual determination, but rather is a conclusion reached by weighing many factual considerations. The board concluded that undue experimentation would be needed to practice the invention on the basis of experimental data presented by Wands. These data are not in dispute. However, Wands and the board disagree strongly on the conclusion that should be drawn from that data.

Factors to be considered in determining whether a disclosure would require undue experimentation have been summarized by the board in *Ex parte Forman*.[24] They include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims.[25]

In order to understand whether the rejection was proper, it is necessary to discuss further the methods for making specific monoclonal antibodies. The first step for making monoclonal antibodies is to immunize an animal. The '145 patent provides a detailed description of procedures for immunizing a specific strain of mice against HBsAg. Next the spleen, an organ rich in lymphocytes, is removed and the lymphocytes are separated from the other spleen cells. The lymphocytes are mixed with myeloma cells, and the mixture is treated to cause a few of the cells to fuse with each other. Hybridoma cells that secrete the desired antibodies then must be isolated from the enormous number of other cells in the mixture. This is done through a series of screening procedures.

The first step is to separate the hybridoma cells from unfused lymphocytes and myeloma cells. The cells are cultured in a medi-

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[18] *Hybritech*, 802 F.2d at 1384, 231 USPQ at 94.

[19] *Id.*; *Atlas Powder Co. v. E.I. DuPont De Nemours & Co.*, 750 F.2d 1569, 1576, 224 USPQ 409, 413 (Fed. Cir. 1984); *In re Angstadt*, 537 F.2d at 502–504, 190 USPQ at 218; *In re Geerdes*, 491 F.2d 1260, 1265, 180 USPQ 789, 793 (CCPA 1974); *Mineral Separation, Ltd. v. Hyde*, 242 U.S. 261, 270–71 (1916).

[20] *Hybritech*, 802 F.2d at 1384, 231 USPQ at 94; *W.L. Gore*, 721 F.2d at 1557, 220 USPQ at 316; *In re Colianni*, 561 F.2d 220, 224, 195 USPQ 150, 153 (CCPA 1977) (Miller, J., concurring).

[21] *In re Angstadt*, 537 F.2d at 504, 190 USPQ at 219.

[22] *In re Jackson*, 217 USPQ at 807.

[23] *See Hybritech*, 802 F.2d at 1384, 231 USPQ at 94; *Atlas Powder*, 750 F.2d at 1576, 224 USPQ at 413.

[24] *Ex parte Forman*, 230 USPQ at 547.

[25] *Id.*; *see In re Colianni*, 561 F.2d at 224, 195 USPQ at 153 (Miller, J., concurring); *In re Rainer*, 347 F.2d 574, 577, 146 USPQ 218, 221 (CCPA 1965).

um in which all the lymphocytes and myeloma cells die, and only the hybridoma cells survive. The next step is to isolate and clone hybridomas that make antibodies that bind to the antigen of interest. Single hybridoma cells are placed in separate chambers and are allowed to grow and divide. After there are enough cells in the clone to produce sufficient quantities of antibody to analyze, the antibody is assayed to determine whether it binds to the antigen. Generally, antibodies from many clones do not bind the antigen, and these clones are discarded. However, by screening enough clones (often hundreds at a time), hybridomas may be found that secrete antibodies against the antigen of interest.

Wands used a commercially available radioimmunoassay kit to screen clones for cells that produce antibodies directed against HBsAg. In this assay the amount of radioactivity bound gives some indication of the strength of the antibody-antigen binding, but does not yield a numerical affinity constant, which must be measured using the more laborious Scatchard analysis. In order to determine which anti-HBsAg antibodies satisfy all of the limitations of appellants' claims, the antibodies require further screening to select those which have an IgM isotype and have a binding affinity constant of at least 10^9 M^{-1}.[26] The PTO does not question that the screening techniques used by Wands were well known in the monoclonal antibody art.

During prosecution Wands submitted a declaration under 37 C.F.R §1.132 providing information about all of the hybridomas that appellants had produced before filing the patent application. The first four fusions were unsuccessful and produced no hybridomas. The next six fusion experiments all produced hybridomas that made antibodies specific for HBsAg. Antibodies that bound at least 10,000 cpm in the commercial radioimmunoassay were classified as "high binders." Using this criterion, 143 high-binding hybridomas were obtained. In the declaration, Wands stated that [27]

> It is generally accepted in the art that, among those antibodies which are binders with 50,000 cpm or higher, there is a very high likelihood that high affinity (Ka [greater than] 10^9 M^{-1}) antibodies will be found. However, high affinity antibodies can also be found among high binders of between 10,000 and 50,000, as is clearly demonstrated in the Table.

The PTO has not challenged this statement.

The declaration stated that a few of the high-binding monoclonal antibodies from two fusions were chosen for further screening. The remainder of the antibodies and the hybridomas that produced them were saved by freezing. Only nine antibodies were subjected to further analysis. Four (three from one fusion and one from another fusion) fell within the claims, that is, were IgM antibodies and had a binding affinity constant of at least 10^9 M^{-1}. Of the remaining five antibodies, three were found to be IgG, while the other two were IgM for which the affinity constants were not measured (although both showed binding well above 50,000 cpm).

Apparently none of the frozen cell lines received any further analysis. The declaration explains that after useful high-affinity IgM monoclonal antibodies to HBsAg had been found, it was considered unnecessary to return to the stored antibodies to screen for more IgMs. Wands says that the existence of the stored hybridomas was disclosed to the PTO to comply with the requirement under 37 C.F.R. §1.56 that applicants fully disclose all of their relevant data, and not just favorable results.[28] How these stored hybridomas are viewed is central to the positions of the parties.

The position of the board emphasizes the fact that since the stored cell lines were not completely tested, there is no proof that any of them are IgM antibodies with a binding affinity constant of at least 10^9 M^{-1}. Thus, only 4 out of 143 hybridomas, or 2.8 percent, were *proved* to fall within the claims. Furthermore, antibodies that were proved to be high-affinity IgM came from only 2 of 10 fusion experiments. These statistics are viewed by the board as evidence that appellants' methods were not predictable or reproducible. The board concludes that Wands' low rate of demonstrated success shows that a person skilled in the art would have to

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[26] The examiner, the board, and Wands all point out that, technically, the strength of antibody-HBsAg binding is measured as *avidity*, which takes into account multiple determinants on the HBsAg molecule, rather than affinity. Nevertheless, despite this correction, all parties then continued to use the term "affinity." We will use the terminology of the parties. Following the usage of the parties, we will also use the term "high-affinity" as essentially synonymous with "having a binding affinity constant of at least 10^9 M^{-1}."

[27] A table in the declaration presented the binding data for antibodies from every cell line. Values ranged from 13,867 to 125,204 cpm, and a substantial proportion of the antibodies showed binding greater than 50,000 cpm. In confirmation of Dr. Wand's statement, two antibodies with binding less than 25,000 cpm were found to have affinity constants greater than 10^9 M^{-1}.

[28] *See Rohm & Haas Co. v. Crystal Chem. Co.*, 722 F.2d 1556, 220 USQ 98 (Fed. Cir. 1983).

engage in undue experimentation in order to make antibodies that fall within the claims.

Wands views the data quite differently. Only nine hybridomas were actually analyzed beyond the initial screening for HBsAg binding. Of these, four produced antibodies that fell within the claims, a respectable 44 percent rate of success. (Furthermore, since the two additional IgM antibodies for which the affinity constants were never measured showed binding in excess of 50,000 cpm, it is likely that these also fall within the claims.) Wands argues that the remaining 134 unanalyzed, stored cell lines should not be written off as failures. Instead, if anything, they represent partial success. Each of the stored hybridomas had been shown to produce a high-binding antibody specific for HBsAg. Many of these antibodies showed binding above 50,000 cpm and are thus highly likely to have a binding affinity constant of at least 10^9 M^{-1}. Extrapolating from the nine hybridomas that were screened for isotype (and from what is well known in the monoclonal antibody art about isotype frequency), it is reasonable to assume that the stored cells include some that produce IgM. Thus, if the 134 incompletely analyzed cell lines are considered at all, they provide some support (albeit without rigorous proof) to the view that hybridomas falling within the claims are not so rare that undue experimentation would be needed to make them.

The first four fusion attempts were failures, while high-binding antibodies were produced in the next six fusions. Appellants contend that the initial failures occurred because they had not yet learned to fuse cells successfully. Once they became skilled in the art, they invariably obtained numerous hybridomas that made high-binding antibodies against HBsAg and, in each fusion where they determined isotype and binding affinity they obtained hybridomas that fell within the claims.

Wands also submitted a second declaration under 37 C.F.R. §1.132 stating that after the patent application was submitted they performed an eleventh fusion experiment and obtained another hybridoma that made a high-affinity IgM anti-HBsAg antibody. No information was provided about the number of clones screened in that experiment. The board determined that, because there was no indication as to the number of hybridomas screened, this declaration had very little value. While we agree that it would have been preferable if Wands had included this information, the declaration does show that when appellants repeated their procedures they again obtained a hybridoma that produced an antibody that fit all of the limitations of their claims.

[1] We conclude that the board's interpretation of the data is erroneous. It is strained and unduly harsh to classify the stored cell lines (each of which was proved to make high-binding antibodies against HBsAg) as failures demonstrating that Wands' methods are unpredictable or unreliable.[29] At worst, they prove nothing at all about the probability of success, and merely show that appellants were prudent in not discarding cells that might someday prove useful. At best, they show that high-binding antibodies, the starting materials for IgM screening and Scatchard analysis, can be produced in large numbers. The PTO's position leads to the absurd conclusion that the more hybridomas an applicant makes and saves without testing the less predictable the applicant's results become. Furthermore, Wands' explanation that the first four attempts at cell fusion failed only because they had not yet learned to perform fusions properly is reasonable in view of the fact that the next six fusions were all successful. The record indicates that cell fusion is a technique that is well known to those of ordinary skill in the monoclonal antibody art, and there has been no claim that the fusion step should be more difficult or unreliable where the antigen is HBsAg than it would be for other antigens.

[2] When Wands' data is interpreted in a reasonable manner, analysis considering the factors enumerated in *Ex parte Forman* leads to the conclusion that undue experimentation would not be required to practice the invention. Wands' disclosure provides considerable direction and guidance on how to practice their invention and presents working examples. There was a high level of skill in the art at the time when the application was filed, and all of the methods needed to practice the invention were well known.

The nature of monoclonal antibody technology is that it involves screening hybridomas to determine which ones secrete antibody with desired characteristics. Practitioners of this art are prepared to screen negative hybridomas in order to find one that makes the desired antibody. No evidence was presented by either party on how many hybridomas would be viewed by those in the art as requiring undue experimentation to screen. However, it seems unlikely that un-

[29] Even if we were to accept the PTO's 2.8% success rate, we would not be required to reach a conclusion of undue experimentation. Such a determination must be made in view of the circumstances of each case and cannot be made solely by reference to a particular numerical cutoff.

due experimentation would be defined in terms of the number of hybridomas that were never screened. Furthermore, in the monoclonal antibody art it appears that an "experiment" is not simply the screening of a single hybridoma, but is rather the entire attempt to make a monoclonal antibody against a particular antigen. This process entails immunizing animals, fusing lymphocytes from the immunized animals with myeloma cells to make hybridomas, cloning the hybridomas, and screening the antibodies produced by the hybridomas for the desired characteristics. Wands carried out this entire procedure three times, and was successful each time in making at least one antibody that satisfied all of the claim limitations. Reasonably interpreted, Wands' record indicates that, in the production of high-affinity IgM antibodies against HBsAG, the amount of effort needed to obtain such antibodies is not excessive. Wands' evidence thus effectively rebuts the examiner's challenge to the enablement of their disclosure.[30]

IV. *Conclusion*

Considering all of the factors, we conclude that it would not require undue experimentation to obtain antibodies needed to practice the claimed invention. Accordingly, the rejection of Wands' claims for lack of enablement under 35 U.S.C. §112, first paragraph, is reversed.

REVERSED

Newman, J., concurring in part, dissenting in part.

A

I concur in the court's holding that additional samples of hybridoma cell lines that produce these high-affinity IgM monoclonal antibodies need not be deposited. This invention, as described by Wands, is not a selection of a few rare cells from many possible cells. To the contrary, Wands states that all monoclonally produced IgM antibodies to hepatitis B surface antigen have the desired high avidity and other favorable properties, and that all are readily preparable by now-standard techniques.

Wands states that his United States Patent No. 4,271,145 describes fully operable techniques, and is distinguished from his first four failed experiments that are referred to in the Rule 132 affidavit. Wands argues that these biotechnological mechanisms are relatively well understood and that the preparations can be routinely duplicated by those of skill in this art, as in *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1380, 231 USPQ 81, 94 (Fed. Cir. 1986), *cert. denied*, 107 S.Ct. 1606 (1987). I agree that it is not necessary that there be a deposit of multiple exemplars of a cell system that is readily reproduced by known, specifically identified techniques.

B

I would affirm the board's holding that Wands has not complied with 35 U.S.C. §112, first paragraph, in that he has not provided data sufficient to support the breadth of his generic claims. Wands' claims on appeal include the following:

> 19. Monoclonal high affinity IgM antibodies immunoreactive with HBsAg determinants, wherein said antibodies are coupled to an insoluble solid phase, and wherein the binding affinity constant of said antibodies for said HBsAg determinants is at least 10^9 M^{-1}.
>
> 26. Monoclonal high affinity IgM antibodies immunoreactive with hepatitis B surface antigen.

Wands states that he obtained 143 "high binding monoclonal antibodies of the right specificity" in the successful fusions; although he does not state how they were determined to be high binding or of the right specificity, for Wands also states that only nine of these 143 were tested.

Of these nine, four (three from one fusion and one from another fusion) were found to have the claimed high affinity and to be of the IgM isotype. Wands states that the other five were either of a different isotype or their affinities were not determined. (This latter statement also appears to contradict his statement that all 143 were "high binding".)

Wands argues that a "success rate of four out of nine", or 44.4%, is sufficient to support claims to the entire class. The Commissioner deems the success rate to be four out of 143, or 2.8%; to which Wands responds with statistical analysis as to how unlikely it is that Wands selected the only four out of 143 that worked. Wands did not, however, prove the right point. The question is whether Wands, by testing nine out of 143 (the Commissioner points out that the randomness of the sample was not established), and finding that four out of the nine had the desired properties, has provided sufficient experimental support for the breadth of the requested claims, in the context that "experi-

[30] *In re Strahilevitz*, 668 F.2d 1229, 1232, 212 USPQ 561, 563 (CCPA 1982).

ments in genetic engineering produce, at best, unpredictable results", quoting from *Ex parte Forman*, 230 USPQ 546, 547 (Bd.Pat.App. and Int. 1986).

The premise of the patent system is that an inventor, having taught the world something it didn't know, is encouraged to make the product available for public and commercial benefit, by governmental grant of the right to exclude others from practice of that which the inventor has disclosed. The boundary defining the excludable subject matter must be carefully set: it must protect the inventor, so that commercial development is encouraged; but the claims must be commensurate with the inventor's contribution. Thus the specification and claims must meet the requirements of 35 U.S.C. §112. *In re Fisher*, 427 F.2d 833, 839, 166 USPQ 18, 23-24 (CCPA 1970).

As the science of biotechnology matures the need for special accommodation, such as the deposit of cell lines or microorganisms, may diminish; but there remains the body of law and practice on the need for sufficient disclosure, including experimental data when appropriate, that reasonably support the scope of the requested claims. That law relates to the sufficiency of the description of the claimed invention, and if not satisfied by deposit, must independently meet the requirements of Section 112.

Wands is not claiming a particular, specified IgM antibody. He is claiming all such monoclonal antibodies in assay for hepatitis B surface antigen, based on his teaching that such antibodies have uniformly reproducible high avidity, free of the known disadvantages of IgM antibodies such as tendency to precipitate or aggregate. It is incumbent upon Wands to provide reasonable support for the proposed breadth of his claims. I agree with the Commissioner that four exemplars shown to have the desired properties, out of the 143, do not provide adequate support.

Wands argues that the law should not be "harsher" where routine experiments take a long time. However, what Wands is requesting is that the law be less harsh. As illustrated in extensive precedent on the question of how much experimentation is "undue", each case must be determined on its own facts. *See, e.g., W.L. Gore & Assocs., Inc. v. Garlock, Inc.*, 721 F.2d 1540, 1557, 220 USPQ 303, 316 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 851 (1984); *In re Angstadt*, 537 F.2d 498, 504, 190 USPQ 214, 218 (CCPA 1976); *In re Cook*, 439 F.2d 730, 734-35, 169 USPQ 298, 302-03 (CCPA 1971).

The various criteria to be considered in determining whether undue experimentation is required are discussed in, for example, *Fields v. Conover*, 443 F.2d 1386, 170 USPQ 276 (CCPA 1971); *In re Rainer*, 347 F.2d 574, 146 USPQ 218 (CCPA 1965); *Ex parte Forman*, 230 USPQ at 547. Wands must provide sufficient data or authority to show that his results are reasonably predictable within the scope of the claimed generic invention, based on experiment and/or scientific theory. In my view he has not met this burden.

Patent and Trademark Office
Trademark Trial and Appeal Board

In re Johanna Farms Inc.

Serial No. 542,343

Decided June 30, 1988

JUDICIAL PRACTICE AND PROCEDURE

1. Procedure — Prior adjudication — In general (§410.1501)

Trademark Trial and Appeal Board's prior decision upholding examiner's refusal to register proposed mark "La Yogurt" does not preclude registration of mark pursuant to subsequent application, since applicant, by presenting survey evidence and consumer letters regarding issue of how purchasers perceive proposed mark, has demonstrated that instant factual situation is different from situation presented in prior proceeding.

TRADEMARKS AND UNFAIR TRADE PRACTICES

2. Types of marks — Non-descriptive — Particular marks (§327.0505)

Term "La Yogurt," with "yogurt" disclaimed, is registrable, since word "yogurt" is common English generic term rather than corruption or misspelling of French word for yogurt, since examining attorney failed to meet burden of showing clear evidence of generic use of mark as whole, and since evidence of record, including survey and consumer letters to applicant, demonstrates that primary significance of "La Yogurt" to majority of relevant public is that of brand name rather than generic term.

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TREATMENT OF RHEUMATOID ARTHRITIS WITH CHIMERIC MONOCLONAL ANTIBODIES TO TUMOR NECROSIS FACTOR α

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Objective. **To evaluate the safety and efficacy of a chimeric monoclonal antibody to tumor necrosis factor α (TNFα) in the treatment of patients with rheumatoid arthritis (RA).**

Methods. **Twenty patients with active RA were treated with 20 mg/kg of anti-TNFα in an open phase I/II trial lasting 8 weeks.**

Results. **The treatment was well tolerated, with no serious adverse events. Significant improvements were seen in the Ritchie Articular Index, which fell from a median of 28 at study entry to a median of 6 by week 6 ($P < 0.001$), the swollen joint count, which fell from 18 to 5 ($P < 0.001$) over the same period, and in the other major clinical assessments. Serum C-reactive protein levels fell from a median of 39.5 mg/liter at study entry to 8 mg/liter at week 6 ($P < 0.001$), and significant decreases were also seen in serum amyloid A and interleukin-6 levels.**

Conclusion. **Treatment with anti-TNFα was safe and well tolerated and resulted in significant clinical and laboratory improvements. These preliminary results support the hypothesis that TNFα is an important regulator in RA, and suggest that it may be a useful new therapeutic target in this disease.**

Despite optimal use of current antirheumatic therapy, the outcome for many patients with rheumatoid arthritis (RA) consists of pain, disability, and premature death (1–3). As a response to the need for more effective and less toxic treatment, and to an increase in our understanding of the pathogenic mechanisms in RA, several groups have used monoclonal antibodies as therapeutic agents in this disease (4–10). Such immunotherapy has been, in most cases, targeted specifically to the T cell, a strategy based on evidence that T cells are involved in the initiation and maintenance of RA (11).

Here, we outline an alternative immunotherapeutic strategy, which involves the use of monoclonal antibodies with specificity for a cytokine, tumor necrosis factor α (TNFα). This approach is based on a body of knowledge regarding the role of cytokines in general, and of TNFα in particular, in the inflammatory process in RA. The first clearly documented study demonstrated the presence of interleukin-1 (IL-1) in RA synovial fluid (12). Subsequently, we and others have reported the presence and local synthesis in

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Table 1. Demographic features of 20 patients with refractory rheumatoid arthritis

Patient	Age/sex	Disease duration (years)	Previous DMARDs*	Concomitant therapy†
1	48/F	7	SSZ, DP, GST, AUR, MTX, AZA, HCQ	Pred. 5 mg
2	63/F	7	SSZ, GST, DP	Para. 1–2 gm
3	59/M	3	AUR, HCQ, GST, MTX, SSZ	Pred. 10 mg, Indo. 225 mg
4	56/M	10	GST, DP, AZA, SSZ	Pred. 12.5 mg, Ibu. 2 gm, Para. 1–2 gm
5	28/F	3	GST, SSZ, DP, AZA	Pred. 8 mg, Para. 1–2 gm, Code. 16 mg
6	40/M	3	SSZ, HCQ, AUR	Nap. 1 gm
7	54/F	7	DP, GST, SSZ, AZA, MTX	Para. 1–2 gm, Code. 16–32 mg
8	23/F	11	HCQ, GST, SSZ, MTX, AZA	Pred. 7.5 mg, Dicl. 100 mg, Para. 1–2 gm, Dext. 100–200 mg
9	51/F	15	GST, HCQ, DP, MTX	Pred. 7.5 mg, Dicl. 125 mg, Para. 1–3 gm
10	47/F	12	SSZ, CYC, MTX	Ben. 4 gm
11	34/F	10	DP, SSZ, MTX	Pred. 10 mg, Para. 1.5 gm, Code. 30–90 mg
12	57/F	12	GST, MTX, DP, AUR	Asp. 1.2 gm
13	51/F	7	SSZ, AZA	Para. 1–4 gm
14	72/M	11	GST, DP, AZA, MTX	Pred. 5 mg, Para. 1–4 gm, Code. 16–64 mg
15	51/F	17	HCQ, DP, SSZ, MTX	Asp. 0.3 gm
16	62/F	16	GST, DP, SSZ, MTX, AZA	Para. 1–4 gm, Code. 16–64 mg
17	56/F	11	SSZ, DP, GST, MTX, HCQ, AZA	Pred. 7.5 mg, Eto. 600 mg, Para. 1–2 gm, Dext. 100–200 mg
18	48/F	14	GST, MTX, DP, SSZ, AUR, AZA	Pred. 7.5 mg, Indo. 100 mg, Para. 1–3 gm
19	42/F	3	SSZ, MTX	Fen. 450 mg, Ben. 6 gm, Code. 30 mg
20	47/M	20	GST, DP, SSZ, AZA	Pred. 10 mg, Para. 1–3 gm

* Disease-modifying antirheumatic drugs (DMARDs) were SSZ = sulfasalazine; DP = D-penicillamine; GST = gold sodium thiomalate; AUR = auranofin; MTX = methotrexate; AZA = azathioprine; HCQ = hydroxychloroquine; CYC = cyclophosphamide.
† Daily doses are shown. Pred. = prednisolone; Para. = paracetamol; Indo. = indomethacin; Ibu. = ibuprofen; Code. = codeine phosphate; Nap. = naprosyn; Dicl. = diclofenac; Dext. = dextropropoxyphene; Ben. = benorylate; Asp. = aspirin; Eto. = etodolac; Fen. = fenbufen.

rheumatoid synovial membrane of many cytokines, including IL-1 (13), TNFα (13,14), IL-6 (15), granulocyte-macrophage colony-stimulating factor (GM-CSF; 16), IL-8 (17), and transforming growth factor β (TGFβ) (18,19).

We have investigated the relationships between these cytokines in RA, using a synovial culture system in which dissociated rheumatoid synovial cells are allowed to spontaneously re-aggregate in vivo. Even in the absence of extrinsic stimulation, such cells express high levels of cytokines and HLA class II molecules (20). Using this system, we showed that production of bioactive IL-1 was abrogated by neutralizing antibodies to TNFα, but not by antibodies to TNFβ or by normal rabbit IgG (21). This occurred in rheumatoid, but not osteoarthritic, cultures and suggested to us that TNFα was of particular importance as a regulatory cytokine. Subsequent analysis reinforced this concept, with the demonstration that another proinflammatory cytokine, GM-CSF, was regulated in the synovial membrane by TNFα (22) and that TNFα receptor expression, necessary for transmitting TNFα signals, was up-regulated in rheumatoid synovium (23,24).

Two recent mouse studies provide further insight into the importance of TNFα in arthritis. Keffer et al (25) described a mouse transgenic for the human TNFα gene, which expressed high levels of human TNFα in vivo and which reproducibly developed arthritis beginning at 4 weeks of age. The disease in these animals could be prevented by administration of monoclonal antibodies to human TNFα. In separate experiments in our own laboratory, we showed that in the type II collagen arthritis model in the DBA/1 mouse, the hamster anti-murine TNF monoclonal antibody TN3.19.2 significantly ameliorated the inflammation and tissue destruction when administered before or after the onset of disease (26).

Based on these considerations, it was of interest to determine the effect of therapy with a chimeric (human IgG1, murine Fv) monoclonal antibody to human TNFα in patients with rheumatoid arthritis. We report here that anti-TNFα therapy was safe and well tolerated, and induced marked improvements in both clinical and laboratory disease measures. These findings are consistent with our postulate concerning the critical role of TNFα in the pathogenesis of RA (27,28), and suggest that TNFα may be a useful therapeutic target in this disease.

Table 2. Changes in clinical assessments following treatment of rheumatoid arthritis patients with cA2*

Week of trial	Morning stiffness, minutes	Pain score, 0–10 cm	Ritchie index, 0–69	Swollen joint count, 0–28	Grip strength, 0–300 mm Hg		IDA, 1–4	Patient's assessment, no. grades improved, 0–3
					Left hand	Right hand		
Screen	135, 0–600	7.4, 4–9.7	23, 4–51	16, 4–28	84, 45–300	96, 57–300	3, 2.3–3.3	NA
0	180, 20–600	7.1, 2.7–9.7	28, 4–52	18, 3–27	77, 52–295	92, 50–293	3, 2–3.5	NA
1	20, 0–180 (<0.001†)	2.6, 0.6–7.8 (<0.001†)	13, 2–28 (<0.001; <0.002†)	13.5, 1–25 (>0.05)	122, 66–300 (>0.05)	133, 57–300 (>0.05)	2, 1.5–3.3 (<0.001†)	1, 1–3
2	15, 0–150 (<0.001†)	3.0, 0.3–6.4 (<0.001†)	13, 1–28 (<0.001†)	11.5, 1–22 (<0.003; <0.02†)	139, 75–300 (<0.03; >0.05†)	143, 59–300 (>0.05)	2, 1.5–3.2 (<0.001†)	1.5, 1–3
3	5, 0–150 (<0.001†)	2.2, 0.2–7.4 (<0.001†)	8, 0–22 (<0.001†)	6, 1–19 (<0.001; <0.002†)	113, 51–300 (>0.05)	142, 65–300 (>0.05)	2, 1.2–3.2 (<0.001†)	2, 1–2
4	15, 0–90 (<0.001†)	1.9, 0.1–5.6 (<0.001†)	10, 0–17 (<0.001†)	6, 0–21 (<0.001; <0.002†)	124, 79–300 (<0.02; >0.05†)	148, 64–300 (<0.03; >0.05†)	1.8, 1.3–2.7 (<0.001†)	2, 1–2
6	5, 0–90 (<0.001†)	1.9, 0.1–6.2 (<0.001†)	6, 0–18 (<0.001†)	5, 1–14 (<0.001†)	119, 68–300 (<0.04; >0.05†)	153, 62–300 (<0.05; >0.05†)	1.7, 1.3–2.8 (<0.001†)	2, 1–2
8	15, 0–60 (<0.001†)	2.1, 0.2–7.7 (<0.001†)	8, 1–28 (<0.001†)	7, 1–18 (<0.001†)	117, 69–300 (<0.03; >0.05†)	167, 53–300 (<0.03; >0.05†)	1.8, 1.5–2.8 (<0.001†)	2, 1–3

* Values are the median, range (*P*) for 20 patients for the initial screen and weeks 0–2, and for 19 patients thereafter. Patient 15 dropped out after week 2 of study. All *P* values versus week 0, by Mann-Whitney test. IDA = Index of Disease Activity; NA = not applicable.
† Adjusted for multiple statistical comparisons.

PATIENTS AND METHODS

Patient selection. Twenty patients were recruited, each of whom fulfilled the American College of Rheumatology (formerly, the American Rheumatism Association) criteria for the diagnosis of RA (29). The clinical characteristics of the patients are shown in Table 1. The study group comprised 15 females and 5 males, with a median age of 51 years (range 23–72), a median disease duration of 10.5 years (range 3–20), and a history of failed therapy with standard disease-modifying antirheumatic drugs (DMARDs) (median number of failed DMARDs 4, range 2–7).

Seventeen patients were seropositive at study entry or had been seropositive at some stage of their disease. All had erosions evident on radiographs of the hands or feet, and 3 had rheumatoid nodules. All patients had active disease at trial entry, as defined by an Index of Disease Activity (IDA) (30) of at least 1.75, together with at least 3 swollen joints, and were classified in anatomic and functional stage II or III (31). The pooled data for each of the clinical and laboratory indices of disease activity at the time of screening for the trial (up to 4 weeks prior to trial entry), and on the day of trial entry itself (week 0), are shown in Tables 2 and 3.

All DMARDs were discontinued at least 1 month prior to trial entry. Patients were allowed to continue taking a nonsteroidal antiinflammatory drug and/or prednisolone (≤12.5 mg/day) during the trial. The dosage of these agents was kept stable for 1 month prior to trial entry and during the course of the trial. No parenteral corticosteroids were allowed during these periods. Simple analgesics were allowed ad libitum.

Patients with other serious medical conditions were excluded from study. Specific exclusions were as follows: serum creatinine >150 μmoles/liter (normal 60–120), hemoglobin (Hgb) <90 gm/liter (normal 120–160 in females, and 135–175 in males), white blood cell (WBC) count <4 × 10^9/liter (normal 4–11 × 10^9/liter), platelet count <100 × 10^9/liter (normal 150–400 × 10^9/liter), and abnormal liver enzyme levels or active pathology noted on chest radiographs.

All patients gave their informed consent for the trial, and approval was granted by the local ethics committee.

Treatment protocol. cA2 is a chimeric human/mouse monoclonal anti-TNFα antibody, consisting of the constant regions of human (Hu)IgG1κ, coupled to the Fv region of a high-affinity neutralizing murine anti-HuTNFα antibody (A2). The antibody was produced by Centocor Inc., by continuous fermentation of a mouse myeloma cell line which had been transfected with cloned DNA coding for cA2, and was purified from culture supernatant by a series of steps involving column chromatography. The chimeric antibody retains specificity for natural and recombinant HuTNFα, and is of high affinity.

The antibody was stored at 4°C in 20-ml vials containing 5 mg of cA2 per milliliter of 0.01*M* phosphate buffered saline in 0.15*M* sodium chloride at a pH of 7.2 and was filtered through a 0.2-μm sterile filter before use. The appropriate amount of cA2 was then diluted to a total volume of 300 ml in sterile saline and administered intravenously via a 0.2-μm in-line filter over a period of 2 hours.

Patients were admitted to the hospital for 8–24 hours for each treatment, and were mobile except during infusions. The trial was of an open, uncontrolled design, with a comparison of 2 treatment schedules. Patients 1–5 and 11–20 received a total of 2 infusions, each consisting of 10 mg/kg of cA2, at entry to the study (week 0) and 14 days later (week 2). Patients 6–10 received a total of 4 infusions of 5 mg/kg at cA2, at entry and on days 4, 8, and 12. The total dose received by the 2 patient groups was therefore the same: 20 mg/kg.

Assessments. *Safety monitoring.* Vital signs were recorded every 15–30 minutes during infusions, and at intervals for up to 24 hours postinfusion. Patients were

Table 3. Changes in laboratory measures following treatment of rheumatoid arthritis patients with cA2*

Week of trial	Hgb, gm/liter	WBC, $\times 10^9$/liter	Platelets, $\times 10^9$/liter	ESR, mm/hour	CRP, mg/liter	SAA, mg/ml	RF, inverse titer
Screen	117, 98–146	7.9, 3.9–15.2	352, 274–631	59, 18–87	42, 9–107	ND	ND
0	113, 97–144	9.0, 4.9–15.7	341, 228–710	55, 15–94	39.5, 5–107	245, 18–1,900	2,560, 160–10,240
1	114, 96–145 (>0.05)	8.5, 3.6–13.6 (>0.05)	351, 223–589 (>0.05)	26, 13–100 (>0.05)	5, 0–50 (<0.001†)	58, 0–330 (<0.001; <0.003†)	ND
2	112, 95–144 (>0.05)	8.2, 4.3–12.7 (>0.05)	296, 158–535 (<0.04; >0.05†)	27, 10–90 (<0.02; >0.05†)	5.5, 0–80 (<0.001; <0.003†)	80, 11–900 (<0.02; <0.04†)	ND
3	110, 89–151 (>0.05)	9.0, 3.7–14.4 (>0.05)	289, 190–546 (<0.03; >0.05†)	27, 12–86 (<0.04; >0.05†)	7, 0–78 (<0.001; <0.002†)	ND	ND
4	112, 91–148 (>0.05)	8.2, 4.7–13.9 (>0.05)	314, 186–565 (>0.05)	23, 10–87 (<0.04; >0.05†)	10, 0–91 (<0.004; <0.02†)	ND	ND
6	116, 91–159 (>0.05)	9.1, 2.9–13.9 (>0.05)	339, 207–589 (>0.05)	23, 12–78 (<0.03; >0.05†)	8, 0–59 (<0.001†)	ND	ND
8	114, 94–153 (>0.05)	7.6, 4.2–13.5 (>0.05)	339, 210–591 (>0.05)	30, 7–73 (>0.05)	6, 0–65 (<0.001†)	ND	480, 40–5,120 (>0.05)

* Values are the median, range (*P*) for 20 patients for the initial screen and weeks 0–2, and for 19 patients thereafter. Patient 15 dropped out after week 2 of study. For rheumatoid factor (RF), only those patients with week 0 titers ≥1:160 in the particle agglutination assay were included (n = 14). All *P* values versus week 0, by Mann-Whitney test. Normal ranges: hemoglobin (Hgb) 120–160 gm/liter in females and 135–175 gm/liter in males; white blood cell (WBC) count 4–11 × 10^9/liter; platelet count 150–400 × 10^9/liter; erythrocyte sedimentation rate (ESR) <15 mm/hour in females and <10 mm/hour in males; C-reactive protein (CRP) <10 mg/liter; serum amyloid A (SAA) <10 mg/ml. ND = not done.

questioned concerning possible adverse events before each infusion and at weeks 1, 2, 3, 4, 6, and 8 of the trial. A complete physical examination was performed at screening and at week 8. In addition, patients were monitored by standard laboratory tests including a complete blood cell count, and levels of C3 and C4 components of complement, IgG, IgM, and IgA, serum electrolytes, creatinine, urea, alkaline phosphatase, aspartate transaminase, and total bilirubin.

Sample times for these tests were between 0800 and 0900 hours (preinfusion) and 1200–1400 hours (24 hours postinfusion). Blood tests subsequent to day 1 were performed in the morning, usually between 0700 and 1200 hours. Urine analysis and culture were also performed at each assessment point.

Response assessment. The patients were assessed for response to cA2 at weeks 1, 2, 3, 4, 6, and 8 of the trial. The assessments were all made between 0700 and 1300 hours by the same observer (AL-F). The following clinical assessments were made: duration of morning stiffness (minutes), pain score (0–10 cm on a visual analog scale), Ritchie Articular Index (maximum score 69) (32), number of swollen joints (28 joint count) (validation described in ref. 33), grip strength (0–300 mm Hg, mean of 3 measurements per hand, by sphygmomanometer cuff), and an assessment of function (the Stanford Health Assessment Questionnaire [HAQ], modified for British patients [34]). In addition, the patients' global assessments of response were recorded using a 5-point scale (worse, no response, fair response, good response, excellent response).

Routine laboratory indicators of disease activity included complete blood cell counts, C-reactive protein (CRP) levels (by rate nephelometry), and the erythrocyte sedimentation rate (ESR; Westergren). Followup assessments were made at monthly intervals after the conclusion of the formal trial period, in order to assess the duration of response.

Analysis of improvement in individual patients was made using two separate indices. First, an IDA was calculated for each time point according to the method of Mallya and Mace (30), with input variables of morning stiffness, pain score, Ritchie Articular Index, grip strength, ESR, and Hgb. The second index calculated was that of Paulus et al (35), which uses input variables of morning stiffness, ESR, joint pain/tenderness, joint swelling, and patient's and physician's global assessments of disease severity.

To calculate the presence (or otherwise) of a response according to this index, two approximations were made to accommodate our data. The swollen joint count used by us (nongraded total of swollen joints of 28 joints assessed), which has been validated (33), was used in place of the more extensive graded count described by Paulus et al, and the patient's and physician's global assessments of *response* recorded by us were approximated to the global assessments of *disease activity* used by Paulus et al (35). In addition to determining response according to these published indices, we selected 6 disease activity assessments of interest (morning stiffness, pain score, Ritchie Articular Index, swollen joint count, ESR, and CRP) and calculated their mean percentage improvement. We have used this value to give an indication of the degree of improvement seen in responding patients.

Immunologic investigations. Rheumatoid factors were measured using the rheumatoid arthritis particle agglutination assay (RAPA) (FujiBerio Inc, Tokyo, Japan), in which titers of 1:160 or greater were considered significant. Rheumatoid factor isotypes were measured by enzyme-linked immunosorbent assay (ELISA) (Cambridge Life Sciences, Ely, UK). Addition of cA2, at concentrations of up to 200 μg/ml, to these assay systems did not alter the assay results (data not shown).

Antinuclear antibodies were detected by immunoflu-

orescence on HEp-2 cells (Biodiagnostics, Upton, UK), and antibodies to extractable nuclear antigens were measured by counterimmunoelectrophoresis with polyantigen extract (Biodiagnostics). Sera positive by immunofluorescence were also screened for antibodies to DNA by the Farr assay (Kodak Diagnostics, Amersham, UK). Anticardiolipin antibodies were measured by ELISA (Shield Diagnostics, Dundee, Scotland). Serum amyloid A (SAA) was measured by sandwich ELISA (Biosource International, Camarillo, CA). Antiglobulin responses to the infused chimeric antibody were measured by an in-house ELISA, using cA2 as a capture reagent.

Cytokine assays. Bioactive TNF was measured in sera using the WEHI 164 clone 13 cytotoxicity assay (36). Total IL-6 was measured in sera using a commercial immunoassay (Medgenix Diagnostics, Brussels, Belgium) and using a sandwich ELISA developed in-house, with monoclonal antibodies provided by Dr. F. di Padova (Basel, Switzerland). Microtiter plates were coated with monoclonal antibody LNI 314-14 at a concentration of 3 μg/ml for 18 hours at 4°C, and blocked with 3% bovine serum albumin in 0.1*M* phosphate buffered saline, pH 7.2. Undiluted sera or standards (recombinant HuIL-6, 0–8.1 μg/ml) were added to the wells in duplicate and incubated for 18 hours at 4°C. Bound IL-6 was detected by incubation with monoclonal antibody LNI 110-14 for 90 minutes at 37°C, followed by biotin-labeled goat anti-murine IgG2b for 90 minutes at 37°C (Southern Biotechnology, Birmingham, AL). The assay was developed using streptavidin–alkaline phosphatase (Southern Biotechnology) and *p*-nitrophenyl phosphate as a substrate, and the optical density read at 405 nm.

Statistical analysis. Data for week 0 versus subsequent time points were compared for each assessment using the Mann-Whitney test. For comparison of rheumatoid factor titers (by RAPA), the data were expressed as dilutions before applying the test.

This was an exploratory study, in which prejudgments about the optimal times for assessment were not possible. Although it has not been common practice to adjust for multiple statistical comparisons in such studies (4–10), a conservative statistical approach would require adjustment of *P* values to take into account analysis at several time points. The *P* values have therefore been presented in two forms: unadjusted, and after making allowance for analysis at multiple time points by use of the Bonferroni adjustment. Where *P* values remained <0.001 after adjustment, a single value only is given. A *P* value of <0.05 is considered significant.

RESULTS

Safety of cA2. The administration of cA2 was exceptionally well tolerated, with no headache, fever, hemodynamic disturbance, allergy, or other acute manifestation. No serious adverse events were recorded during the 8-week trial. Two minor infective episodes were recorded, each "possibly related" to cA2: patient 15 presented at week 2 with clinical features of bronchitis. Sputum culture grew only normal commensals. She had a history of smoking and of a similar illness 3 years previously. The illness responded promptly to treatment with amoxicillin, but her second cA2 infusion was withheld and the data for this patient are therefore not analyzed beyond week 2. Patient 18 showed significant bacteriuria on routine culture at week 6 ($>10^5$/ml; lactose-fermenting coliform), but was asymptomatic. This condition also responded promptly to amoxicillin.

Routine analysis of blood samples showed no consistent adverse changes in hematologic parameters, renal function, liver function, or levels of C3, C4, or immunoglobulins during the 8 weeks of the trial. Four minor, isolated, and potentially adverse laboratory disturbances were recorded. Patient 2 experienced a transient rise in blood urea levels, from 5.7 mmoles/liter to 9.2 mmoles/liter (normal 2.5–7), with no change in serum creatinine. This change was associated with the temporary use of a diuretic, which had been prescribed for a non-rheumatologic disorder. The value normalized within 1 week and was classified as "probably not related" to cA2.

Patient 6 experienced a transient fall in the peripheral blood lymphocyte count, from 1.6×10^9/liter to 0.8×10^9/liter (normal 1.0–4.8). This abnormality was not seen at the next sample point (2 weeks later), was not associated with any clinical manifestations, and was classified as "possibly related" to cA2. Patients 10 and 18 developed elevated titers of anti-DNA antibodies at weeks 6 and 8 of the trial. Elevated anticardiolipin antibodies were also detected in patient 10. Both patients had a preexisting positive antinuclear antibody titer, and patient 10 had a history of borderline lymphocytopenia and high serum IgM. There were no clinical features of systemic lupus erythematosus, and the laboratory changes were judged "probably related" to cA2.

Efficacy of cA2. The pattern of response for each of the clinical assessments of disease activity and the derived IDA are shown in Table 2. All clinical assessments showed improvement following treatment with cA2, with maximal responses from week 3. Duration of morning stiffness decreased from a median of 180 minutes at study entry (week 0) to 5 minutes at week 6 ($P < 0.001$ by Mann-Whitney test, adjusted), representing a 97% improvement. The pain score decreased from 7.1 to 1.9 over the same period ($P < 0.001$, adjusted), representing an improvement of 73%. Similarly, the Ritchie Articular Index improved from 28 to 6 at week 6 ($P < 0.001$, adjusted; 79% improvement), and the swollen joint count decreased from 18

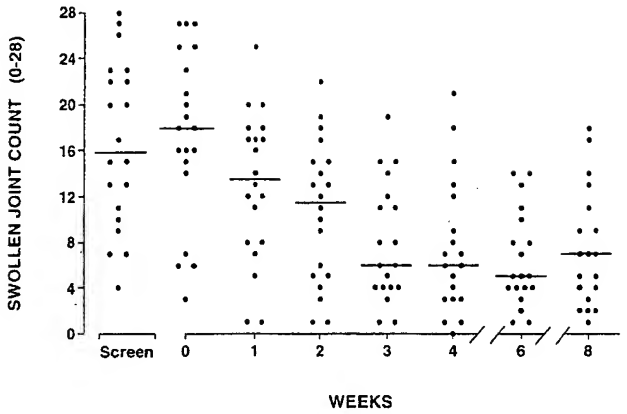

Figure 1. Swollen joint counts (maximum 28), as recorded by a single observer, in 20 patients with rheumatoid arthritis treated with cA2. The screening time point was within 4 weeks of entry to the study (week 0); data from patient 15 were not included after week 2 (dropout). Significance of the changes, relative to week 0, were determined by Mann-Whitney test (adjusted): $P > 0.05$ at week 1, $P < 0.02$ at week 2, $P < 0.002$ at weeks 3 and 4, and $P < 0.001$ at weeks 6 and 8. Bars show median values.

to 5 ($P < 0.001$, adjusted; 72% improvement). The individual swollen joint counts for all time points are shown in Figure 1.

Grip strength also improved; the median grip strength rose from 77 mm Hg (left) and 92 mm Hg (right) at week 0 to 119 (left) and 153 (right) at week 6 ($P < 0.04$ and $P < 0.05$, left and right hands, respectively; $P > 0.05$ both hands, adjusted for multiple comparisons). The IDA has a range of 1 (normal) to 4 (severe disease activity). The IDA showed a decrease from a median of 3 at study entry to 1.7 at week 6 ($P < 0.001$, adjusted). Patients were asked to grade their responses to cA2 using a 5-point scale. No patient recorded a response of "worse" or "no change" at any point in the trial. "Fair," "good," and "excellent" responses were classified as improvements of 1, 2, and 3 grades, respectively. At week 6, there was a median of 2 grades of improvement (Table 2).

We also measured changes in the patients' functional capacity, using the HAQ, as modified for British patients (range 0–3). The median (range) HAQ score improved from 2 (0.9–3) at study entry to 1.1 (0–2.6) by week 6 ($P < 0.001$ and $P < 0.002$ adjusted).

The changes in the laboratory values which reflect disease activity are shown in Table 3. The most rapid and impressive changes were seen in serum CRP levels, which fell from a median of 39.5 mg/liter at week 0 (normal <10) to 8 mg/liter by week 6 of the trial ($P < 0.001$, adjusted), representing an improvement of 80%. Of the 19 patients with elevated CRP at study entry, 17 showed decreases to the normal range at some point during the trial. The improvement in CRP was maintained in most patients over the assessment period (Table 3 and Figure 2); the exceptions with high values at 4 and 6 weeks tended to be those with the highest starting values (data not shown).

The ESR also showed improvement, with a fall from 55 mm/hour at study entry (normal <10 in males and <15 in females) to 23 mm/hour at week 6 ($P < 0.03$ and $P > 0.05$ adjusted; 58% improvement). SAA levels were elevated in all patients at trial entry, and fell from a median of 245 mg/ml (normal <10) to 58 mg/ml at week 1 ($P < 0.003$ adjusted; 76% improvement) and to 80 mg/ml at week 2 ($P < 0.04$, adjusted). No significant changes were seen in Hgb level, WBC count, or platelet count at week 6, although the platelet count did improve at weeks 2 and 3 compared with trial entry (Table 3).

The response data were also analyzed for each patient individually (not shown). The majority of patients had their best overall responses at week 6, at which time 13 assessed their responses as "good" while 6 assessed their responses as "fair." Eighteen of the 19 patients who completed the treatment schedule achieved an improvement in the IDA of 0.5 or greater at week 6, and 10 achieved an improvement of 1.0 or greater. All patients achieved a response at week 6

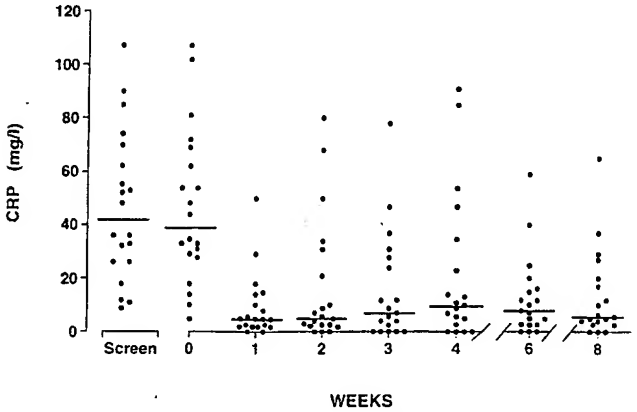

Figure 2. Serum C-reactive protein (CRP) levels (normal 0–10 mg/liter), as measured by nephelometry, in 20 patients with rheumatoid arthritis treated with cA2. The screening time point was within 4 weeks of entry to the study (week 0); data from patient 15 were not included after week 2 (dropout). Significance of the changes, relative to week 0, were determined by Mann-Whitney test (adjusted): $P < 0.001$ at week 1, $P < 0.003$ at week 2, $P < 0.002$ at week 3, $P < 0.02$ at week 4, and $P < 0.001$ at weeks 6 and 8. Bars show median values.

according to the index described by Paulus et al (35). At week 6, all patients showed a mean improvement of 30% or greater in the 6 selected measures of disease activity (see Patients and Methods), with 18 of the 19 patients showing a mean improvement of 50% or greater (data not shown).

Although the study was primarily designed to assess the short-term effects of cA2 treatment, followup clinical and laboratory data are available for those patients followed for sufficient time (n = 12). The duration of response in these patients, defined as the duration of a 30% (or greater) mean improvement in the 6 selected disease activity measures, was variable, ranging from 8 weeks to 25 weeks (median 14) (data not shown).

Comparison of the clinical and laboratory data for patients treated with 2 infusions of cA2 (each at 10 mg/kg) versus those treated with 4 infusions (each at 5 mg/kg) showed no significant differences in the rapidity or extent of response (data not shown).

Immunologic investigations and cytokines. Measurement of rheumatoid factor by RAPA showed 14 patients with significant titers (≥1:160) at trial entry. Of these, 6 patients showed a decrease of at least 2 titers on treatment with cA2, while the remaining patients showed a change of 1 titer or less. No patient showed a significant increase in rheumatoid factor titer during the trial (data not shown). The median titer in the 11 patients decreased from 1:2,560 at entry to 1:480 by week 8 ($P > 0.05$) (Table 3). Specific rheumatoid factor isotypes were measured by ELISA, and showed decreases in the 6 patients whose RAPA had declined significantly, as well as in some other patients (data not shown). Median values for the 3 isotypes in the 14 patients seropositive at trial entry were 119, 102, and 62 IU/ml (IgM, IgG, and IgA isotypes, respectively) and at week 8 were 81, 64, and 46 IU/ml ($P > 0.05$).

We tested sera from patients 1–9 for the presence of bioactive TNF, using the WEHI 164 clone 13 cytotoxicity assay (36). In 8 patients, serum samples spanning the entire trial period were tested; while for patient 9, only 3 samples (1 pretrial, 1 intermediate, and the last available sample) were tested. The levels of bioactive TNF were below the limit of sensitivity of the assay in the presence of human serum (1 pg/ml) (data not shown).

Since production of CRP and SAA are thought to be regulated in large part by IL-6, we also measured serum levels of this cytokine, using 2 different assays which measure total IL-6. In the Medgenix assay, IL-6 was significantly elevated in 17 of the 20 patients at study entry. In this group, levels fell from 60 pg/ml (range 18–500) to 40 pg/ml (range 0–230) at week 1 ($P > 0.05$) and to 32 pg/ml (range 0–210) at week 2 ($P < 0.005$ and $P < 0.01$, adjusted). These results were supported by measurement of serum IL-6 in the first 16 patients in a separate ELISA developed in-house. IL-6 was detectable in 11 of these samples, with median (range) levels falling from 210 pg/ml (25–900) at entry to 32 pg/ml (0–1,700) at week 1 ($P < 0.02$ and $P < 0.04$, adjusted) and to 44 pg/ml (0–240) at week 2 ($P < 0.02$ and $P < 0.03$, adjusted).

We tested sera from patients 1–10 for the presence of antiglobulin responses to the infused chimeric antibody, but none were detected (data not shown). In many patients, however, cA2 was still detectable in serum samples taken at week 8 (data not shown) and this may have interfered with the ELISA.

DISCUSSION

This is the first report describing the administration of anti-TNFα antibodies for treatment of human autoimmune disease. Many cytokines are produced in rheumatoid synovium, but we chose to specifically target TNFα because of mounting evidence that it was a major molecular regulator in RA (21,22,26–28). The study results presented here support that view and allow 3 important conclusions to be drawn.

First, treatment with cA2 was safe and the infusion procedure was well tolerated. Although fever, headache, chills, and hemodynamic disturbance have all been reported following treatment with anti-CD4 or anti-CDw52 in RA (6,10), such features were absent in our patients. Also notable was the absence of any allergic event despite repeated treatment with the chimeric antibody, although the interval between initial and repeat infusions may have been too short to allow maximal expression of any antiglobulin response. The continuing presence of circulating cA2 at the conclusion of the trial may have precluded detection of antiglobulin responses, but also indicated that any such responses were likely to be of low titer and/or affinity. Although we recorded 2 episodes of infection among the study group, these were minor and the clinical courses were unremarkable. TNFα has been implicated in the control of *Listeria* and other infections in mice (37), but our limited experience does not suggest an increased risk of infection after TNFα blockade in humans.

The second conclusion concerns the clinical

efficacy of cA2. The patients we treated had longstanding, erosive, and for the most part, seropositive disease, and therapy with several standard DMARDs had failed. Despite this, the major clinical assessments of disease activity and outcome (morning stiffness, pain score, Ritchie articular index, swollen joint count, and HAQ score) showed statistically significant improvement, even after adjustment for multiple comparisons. All patients graded their response as at least "fair," with the majority grading it as "good." In addition, all achieved a response according to the criteria of Paulus et al and showed a mean improvement of at least 30% in 6 selected disease activity measures. The design of the trial does not allow these results to be attributed to the action of cA2 alone. However, the extent of the clinical improvements, their consistency throughout the study group, and the parallel changes in laboratory indices of disease activity (see below) are encouraging.

The improvements in clinical assessments following treatment with cA2 appear to be at least as good as those reported following treatment of similar patients with antileukocyte antibodies (6,10), although firm conclusions concerning each of these agents will require controlled, blinded studies. The two therapeutic approaches may already be distinguished, however, by their effects on the acute-phase response, since in several studies of antileukocyte antibodies, no consistent improvements in CRP or ESR were seen (4–6,8,10). In contrast, treatment with cA2 resulted in significant decreases in serum CRP and SAA values, with normalization of values in many patients. The changes were rapid and marked, and in the case of CRP, sustained for the duration of the study (Table 3). The decreases in ESR were less marked, achieving statistical significance only when unadjusted for the number of comparisons (Table 3).

These results are consistent with current concepts that implicate TNFα in the regulation of hepatic acute-phase protein synthesis, either directly, or by control of other, secondary, cytokines such as IL-6 (38,39). To investigate the mechanism of control of the acute-phase response in our patients, we measured serum TNFα and IL-6 before and after cA2 treatment. Bioactive TNFα was not detectable in sera obtained at baseline or subsequently. In view of previous reports of variability between different immunoassays in the measurement of cytokines in biologic fluids (40), we used 2 different assays for IL-6, and both demonstrated significant decreases in serum IL-6 levels by week 2. These findings support the other objective laboratory changes induced by cA2, and provide in vivo evidence that TNFα may be a regulatory cytokine for IL-6 in this disease. Among the other laboratory tests performed, levels of rheumatoid factors fell significantly in 6 patients.

The mechanism of action of cA2 leading to the clinical responses outlined above was not established in this study. Neutralization of TNFα may have a number of beneficial consequences, including a reduction in the local release of cytokines such as IL-6 and other inflammatory mediators, and modulation of synovial endothelial/leukocyte interactions. cA2 may also bind directly to synovial inflammatory cells expressing membrane TNFα, with subsequent in situ cell lysis. Further studies should establish which actions of cA2 may be clinically important.

The results obtained in this small series have important implications, both scientifically and clinically. At the scientific level, the ability of the neutralizing antibody, cA2, to reduce acute-phase protein synthesis, reduce the production of other cytokines such as IL-6, and significantly improve the clinical state demonstrates that it is possible to interfere with the cytokine network in a useful manner without untoward effects. Due to the many functions and overlapping effects of cytokines such as IL-1 and TNFα, and the fact that cytokines induce the production of other cytokines and of themselves, there had been some pessimism as to whether targeting a single cytokine in vivo would have any beneficial effect (41,42). This view is clearly refuted. On the clinical side, the results of short-term treatment with cA2 are encouraging, and suggest that TNFα may be a useful new therapeutic target in RA.

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Inhibition of immunoreactive tumor necrosis factor-alpha by a chimeric antibody in patients infected with human immunodeficiency virus type 1.

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Walker RE; Spooner KM; Kelly G; McCloskey RV; Woody JN; Falloon J; Baseler M; Piscitelli SC; Davey RT Jr; Polis MA; Kovacs JA; Masur H; Lane HC; National Institute of Allergy and Infectious Diseases, Critical; Care Medicine Department, National Institutes of Health,; Bethesda, MD 20892, USA.

Abstract: Tumor necrosis factor-alpha (TNF-alpha), a proinflammatory cytokine known to stimulate human immunodeficiency virus type 1 (HIV-1) replication, has been implicated in the pathogenesis of HIV-1 infection. Inhibition of TNF-alpha by a chimeric humanized monoclonal antibody, cA2, was investigated in 6 HIV-1-infected patients with CD4 cell counts < 200/mm3. Two consecutive infusions of 10 mg/kg 14 days apart were well tolerated, and a prolonged serum half-life for cA2 (mean, 257 +/- 70 h) was demonstrated. Serum immunoreactive TNF-alpha concentrations fell from a mean prestudy value of 6.4 pg/mL (range, 4.2-7.9) to 1.1 pg/mL (range, 0.5-2.2) 24 h after the first infusion and returned to baseline within 7-14 days. A similar response was seen after the second infusion. No consistent changes in CD4 cell counts or plasma HIV RNA levels were observed over 42 days. Future studies evaluating the therapeutic utility of long-term TNF-alpha suppression using anti-TNF-alpha antibodies are feasible and warranted.

Keywords: Acquired Immunodeficiency Syndrome/BLOOD/*IMMUNOLOGY Adult Animal Antibodies/*THERAPEUTIC USE Antibodies, Monoclonal/*THERAPEUTIC USE Chimeric Proteins/PHARMACOKINETICS/*THERAPEUTIC USE Female Human *HIV-1 Male Mice Recombinant Proteins/PHARMACOKINETICS/THERAPEUTIC USE Tumor Necrosis Factor/*ANTAGONISTS & INHIB/IMMUNOLOGY JOURNAL ARTICLE
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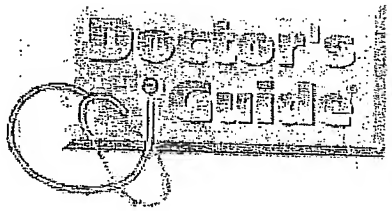

Medical & Other News

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Title: **New Monoclonal Antibody Effective Treatment For Crohn's Disease Therapy**
URL: http://www.pslgroup.com/dg/2802E.htm
Doctor's Guide
May 13, 1997

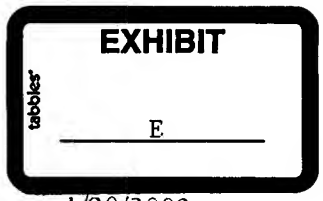
WASHINGTON, and MALVERN, Pa., May 13, 1997 -- Statistically significant results were released yesterday from two controlled clinical studies testing cA2(TM) (infliximab), a monoclonal antibody, in the treatment of Crohn's disease, a chronic disorder characterized by inflammation of the gastrointestinal tract. Data from both trials show that treatment with cA2 can have a beneficial effect on both the severity and number of symptoms associated with Crohn's disease.

"This kind of clinical response in Crohn's disease is unprecedented," said Stephan Targan, M.D., principal investigator and Director of the Inflammatory Bowel Disease Center at Cedars-Sinai Medical Center in Los Angeles, "and provides compelling evidence of the potential of cA2 in the treatment of Crohn's disease."

The results of these trials, which were conducted in 18 centers in North America and Europe, were announced today during Digestive Disease Week in Washington, DC. Digestive Disease Week is sponsored by the American Association for the Study of Liver Diseases, the American Gastroenterological Association, the American Society for Gastrointestinal Endoscopy and The Society for Surgery of the Alimentary Tract.

Last year, during Digestive Disease Week, Centocor released data showing a statistically significant improvement in disease activity following a single infusion of cA2. In the initial study, 65 percent of patients treated with cA2 achieved a clinical response and 33 percent of patients went into remission within four weeks of the start of treatment.

In the extension phase of this study, known as T16, which is being reported today, additional cA2 treatments were demonstrated to maintain Crohn's disease patients in clinical remission as measured by the CDAI, the Crohn's disease activity index.



In the initial phase of the T16 trial, the median CDAI of treated patients dropped from 312 to 125 eight weeks after a single cA2 infusion. Following four additional infusions, given eight weeks apart in the most recent phase of the T16 trial, cA2 maintained the CDAI reduction, with median CDAI eight weeks following the final treatment at 117 (CDAI<150 constitutes disease remission).

Data from the second trial, named T20, indicate that cA2 may be a valuable treatment for enterocutaneous fistulae, a painful, debilitating complication of Crohn's disease in which extensions occur between the bowel and the skin, mostly in the perianal area, causing drainage of mucous and/or fecal material. In this trial, approximately two-thirds of participants experienced closure of at least 50 percent of their fistulae.

In both clinical trials, onset of cA2 clinical benefit was rapid with the vast majority of responders achieving response within two weeks. In addition, cA2 was generally well tolerated in these two trials. "We have been following these studies with great interest," said Richard P. MacDermott, M. D., Immediate Past Chairperson, National Scientific Advisory Committee, Crohn's & Colitis Foundation of America (CCFA). "The results are very encouraging. It is possible that an important new therapy for Crohn's disease may be on the horizon."

In the T16 study, 73 patients who showed a clinical response eight weeks after their initial infusion of cA2 were re-randomized at week 12 to further treatment with cA2 or placebo, and infused every eight weeks for a total of four additional infusions. Those patients re-randomized to cA2 continued to experience an improvement in symptoms from baseline assessment and the percentage of patients achieving clinical remission was maintained at approximately 60 percent during the re-treatment period.

Those patients who responded to their initial infusion of cA2 and then received placebo in the re-treatment phase of the study, experienced a gradual decline in clinical effect over time. However, 19 percent of the placebo group were still in remission 48 weeks after their initial cA2 infusion.

The second study, T20, was conducted with 94 patients with draining enterocutaneous fistulae. Following a series of three cA2 infusions given two and four weeks apart, two-thirds of patients experienced closure of at least 50 percent of their fistulas (P=0.002). These patients had previously failed to respond adequately to treatment with combinations of corticosteroids, methotrexate, 6-MP/azathioprine, aminosalicylates, or antibiotics. These underlying therapies were given in conjunction with the cA2 infusions in this study. "cA2 is the first drug to ever demonstrate statistical significance in a controlled trial to close fistulas," according to Daniel Present, M.D., principal investigator and Clinical Professor of Medicine at Mount Sinai.

cA2, a monoclonal antibody, is the first of a revolutionary class of agents being studied for Crohn's disease. It is a well-tolerated, highly selective treatment that blocks activity of a key inflammatory mediator called tumor necrosis factor or TNF. cA2 is also being studied for treatment of rheumatoid arthritis.

Centocor is a biotechnology company whose mission is to develop and commercialize novel therapeutic and diagnostic products and services that solve critical needs in human health care. The company concentrates on research and development, manufacturing and market development, with a primary technology focus on monoclonal antibodies and DNA-based products.

More information about the company and cA2 can be found on Centocor's home page located at the following address. For more information about Crohn's disease or ulcerative colitis, a related disorder, contact the Crohn's & Colitis Foundation of America, at 1-800-343-3637 (website: http://www.ccfa.org).

Return to News Story Page

-continued

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

ATCGGACGTGGACGTGCAGA 20

What is claimed is:

1. A chimeric antibody comprising at least part of a human immunoglobulin constant region and at least part of a non-human immunoglobulin variable region, said antibody capable of binding an epitope specific for human tumor necrosis factor TNFα, wherein the non-human immunoglobulin variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 3 and SEQ ID NO: 5.

2. An immunoassay method for detecting human TNF in a sample, comprising:

(a) contacting said sample with an antibody according to claim 1, or a TNF binding fragment thereof, in detectably labeled form; and

(b) detecting the binding of the antibody to said TNF.

3. A chimeric antibody comprising at least part of a human immunoglobulin constant region and at least part of a non-human immunoglobulin variable region, said antibody capable of binding an epitope specific for human tumor necrosis factor TNFα, wherein the non-human immunoglobulin variable region comprises a polypeptide encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 2 and SEQ ID NO: 4.

4. An immunoassay method for detecting human TNF in a sample, comprising:

(a) contacting said sample with an antibody according to claim 3, or a TNF binding fragment thereof, in detectably labeled form; and

(b) detecting the binding of the antibody to said TNF.

5. A chimeric antibody, comprising two light chains and two heavy chains, each of said chains comprising at least part of a human immunoglobulin constant region and at least part of a non-human immunoglobulin variable region, said variable region capable of binding an epitope of human tumor necrosis factor hTNFα, wherein said light chains comprise variable regions comprising SEQ ID NO: 3 and said heavy chains comprise variable regions comprising SEQ ID NO: 5.

6. A chimeric antibody according to claim 5, wherein the human immunoglobulin constant region is an IgG1.

7. A chimeric antibody comprising at least part of a human IgG1 constant region and at least part of a non-human immunoglobulin variable region, said antibody capable of binding an epitope specific for human TNFα, wherein the non-human immunoglobulin variable region comprises a polypeptide encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 2 and SEQ ID NO: 4.

8. A polypeptide comprising the amino acid sequence of SEQ ID NO: 3, wherein said polypeptide binds to hTNFα and competitively inhibits the binding of monoclonal antibody cA2 to hTNFα.

9. A polypeptide comprising the amino acid sequence of SEQ ID NO: 5, wherein said polypeptide binds to hTNFα and competitively inhibits the binding of monoclonal antibody cA2 to hTNFα.

* * * * *